Vol. LII *June, 1927* *No. 6*

BIOLOGICAL BULLETIN

OBSERVATIONS ON THE LIFE-HISTORY OF *AMŒBA PROTEUS*.[1]

H. R. HULPIEU
IN COLLABORATION WITH
D. L. HOPKINS,

Zoölogical Laboratory, The Johns Hopkins University.

There is still considerable diversity of opinion concerning the life-history of *Amœba proteus*. Some hold that it reproduces exclusively by binary fission; others do not agree with this.

Carter ('56) asserts that he observed the nucleus in a given specimen, break up into several small nuclei each of which associated itself with a bit of cytoplasm; and that he then saw the membrane of the amœba break and the small nuclei with their surrounding cytoplasm escape into the external medium and move away by amœboid movement.

Wallich ('63), Scheel ('99), Calkins ('05 and '07), Metcalf ('10), Hausman ('20), and Taylor ('24) maintain that they made similar observations. Scheel and Calkins contend that the amœbæ encyst before the nucleus breaks up and Calkins holds that there is a multinucleated generation which ends by sexual activity. Calkins says, "The fertilized cell of Amœba (unknown at present) gives rise to a young amœboid organism formerly known as *Amœba proteus*." Later ('07) he sectioned the amœbæ on which these observations were made and maintains that he found a process of internal fertilization very similar to endomyxis.

Metcalf ('10) asserts that there are two methods of reproduction in which fragmentation occurs; one in which the parent amœba breaks up liberating minute amœboid forms which develop into large amœbæ; another in which small amœboid forms are liberated from the parent by a gemmule formation. These

[1] These observations were made while the authors were working under Dr. S. O. Mast, to whom they are very grateful for timely criticism and helpful suggestions.

amœboid forms he asserts develop flagella, then fuse in pairs, after which the zygotes thus formed develop into large amœbæ.

Taylor gives a detailed description of the nuclear processes accompanying fragmentation. She maintains that the small fragments as they emerge from the parent amœba are cysts which hatch out after varying lengths of time.

All of these observations indicate that *Amœba proteus* at times fragments, forming numerous small amœboid forms, and they appear to indicate that these small forms develop into the large forms which are usually studied. Hausman contends that he actually observed the transformation. There are, however, some prominent investigators, who hold that the evidence presented is not conclusive; for example, Schaeffer, ('26, p. 111.) who says, "We find no proof that the life cycle of the common large ameba includes more than . . . reproduction by fission." The following observations have a definite bearing on this question:

During the last two years we have had under close observation numerous cultures of *Amœba proteus*, started from a few specimens collected in the summer of 1924. During this time it was repeatedly noticed in various cultures that the amœbæ multiplied very rapidly for a while, then suddenly practically disappeared. At first these cultures were all discarded, except a few which were saved for the other protozoa living in them. These were set aside and observed from time to time. After about a week, large numbers of minute amœbæ were found in some of them, and several weeks later numerous large amœbæ.

In further observations it was discovered in several cultures of large amœbæ, (1) that after a period of rapid multiplication by fission the specimens became increasingly more sluggish, darker and more granular in appearance; (2) that they began to decrease in number; and (3) that as the large amœbæ decreased small ones appeared and increased. This did not occur in all cultures, for in some all amœbæ died.

This disappearance of the large amœbæ was observed to occur in several cultures as the solution changed from acid to alkaline in reaction (pH 6.8 to 7.4) or *vice versa*. In other cultures, however, there was no such change, in these the solution remained almost constant as to bacterial content, clearness, amount of food and hydrogen-ion concentration.

Similar results were obtained in cultures on hollow ground slides. All of these cultures consisted of a few drops of filtered culture fluid. One large amœba was put into each of about half of them, and none in the rest. Numerous small amœboid forms appeared in many of the former but in none of the latter. A description of a typical experiment follows:

A large sluggish individual was selected, washed three times in about 5 cc. of distilled water and placed on a hollow ground slide in culture fluid which had been passed through number 50 filter paper. This solution contained no amœbæ that could be seen under a highpower dissecting binocular, or a 1.9 mm. water-immersion objective. The amœba was observed from time to time. The second day after it had been put into the culture fluid on the slide, it became extremely sluggish and remained so. The following morning it had disappeared and in the region where it had been, there were from 150 to 200 minute amœboid forms, about 10 μ in length. A drop of fresh sterile hay infusion was now added every other day for about a week and it could clearly be seen that the small amœboid forms were becoming distinctly larger. Unfortunately, at the end of this time too much solution was added and the forms died.

Following these experiments several amœbæ were isolated and closely watched for a long period of time. The process of fragmentation was actually observed to occur in a number of cases.

The results obtained seem to prove conclusively that *Amœba proteus* at times breaks up into small amœboid forms but they do not prove that these small forms develop into large ones. The evidence presented in the following paragraphs appears, however, to prove this.

On February 19, 1925, two grams of timothy hay were added to 1000 cc. of spring water and boiled for ten minutes. While still hot some of the fluid was poured into a sterile 100 cc. pyrex flask. This was then plugged tightly with cotton. When the flask had cooled a few drops of old culture fluid, which had been passed through number 50 filter paper, were added. The flask was then again plugged with cotton and allowed to stand for a week. Then this culture, free from amœbæ as shown by careful observation, was inoculated by one amœba which had been washed in several

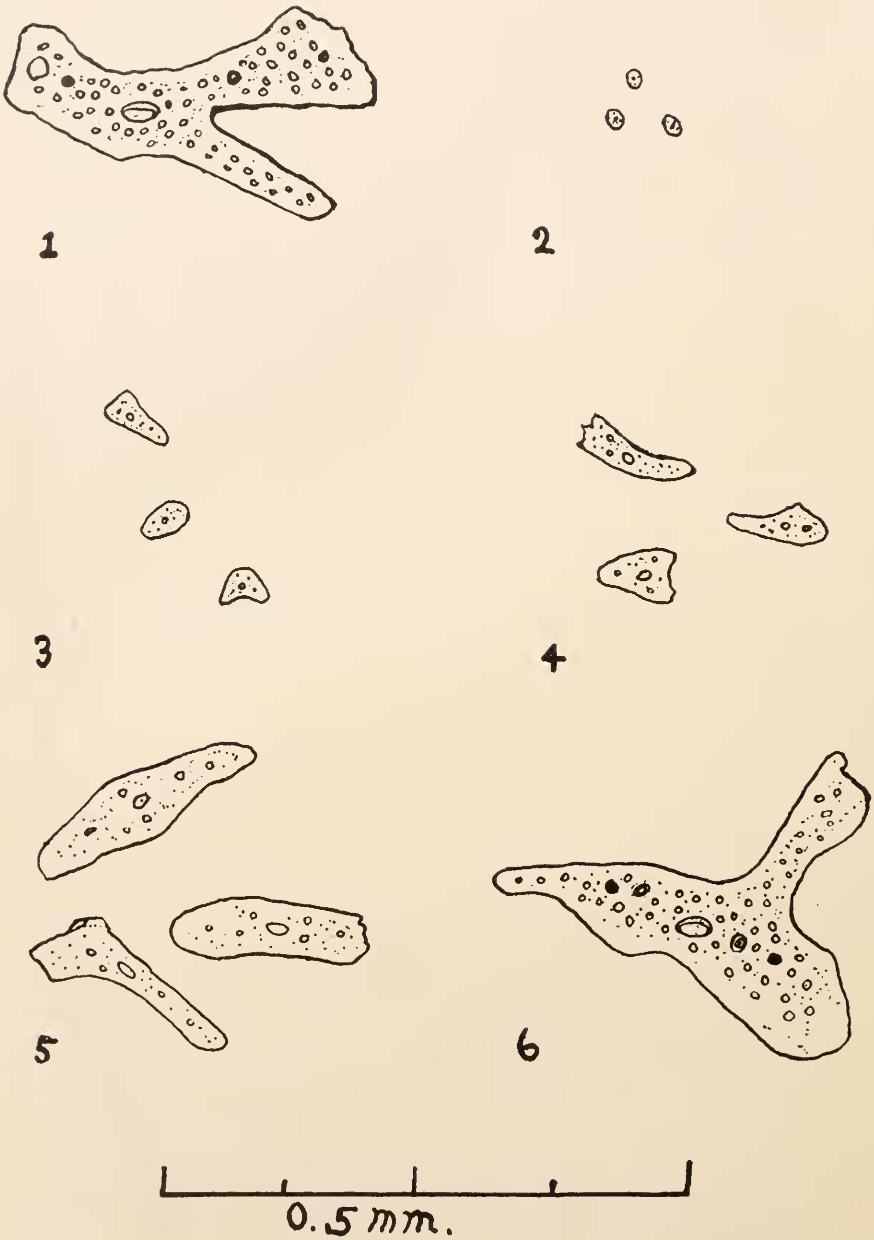

FIG. 1. Camera lucida sketches of some of the largest amœbæ in a culture at different time intervals, showing growth of small amœbæ into large amœbæ. 1, one of the original amœbæ which fragmented, giving rise to small amœbæ; 2,

changes of distilled water. After this there was added each day for food about 5 cc. of fresh sterile culture fluid like the original culture fluid. All pipettes used in handling the amœba and the culture fluid were repeatedly sterilized in order to eliminate contamination.

The culture was poured into a sterile shallow dish and thoroughly examined every few days. It was found that the individual multiplied by fission until in about two weeks there were perhaps fifty large amœbæ in the culture. Then fission appeared to cease and the amœbæ assumed a dark granular color, after which they began rapidly to disappear so that by the end of the third week there were no large amœbæ present. If there had been one large amœba left it could not have escaped the rigid inspection to which the culture was subjected. The culture was now set aside for a week, after which it was again examined. It now contained numerous small amœbæ the length of which varied from 10 to 40 μ, but no large amœbæ. For over a month following this the culture was repeatedly closely observed. The amœbæ increased in size until by the last of April there were numerous specimens which measured 300–600 μ in length.

On September 17, 1926, fourteen cultures made up in a modified Ringer's solution were inoculated with amœbæ from an ordinary spring water culture after being washed in six changes of the Ringer's solution. At the end of four days all of the large amœbæ had disappeared and soon after this small amœboid forms were observed in great numbers. From this time on the cultures were thoroughly examined at the end of each week and after each examination camera lucida sketches were made of the largest amœbæ found in each culture. Some of these sketches are herewith reproduced (Fig. 1). They show that the amœboid forms increased from an approximate average volume of 400 cubic micra to one of 250,000 cubic micra and that this increase required two months. During this time, however, it was observed that these small amœbæ occasionally divided (Fig. 2). All of

small amœbæ produced by fragmentation of original large amœbæ; 3, largest amœbæ in culture ten days after fragmentation; 4, largest amœbæ in culture four weeks after fragmentation; 5, largest amœbæ in culture six weeks after fragmentation; 6, largest amœbæ in culture eight weeks after fragmentation.

these cultures and the one mentioned in the preceding paragraph, were thoroughly examined at least twenty-five times during the two months growth period, and no large amœbæ could be dis-

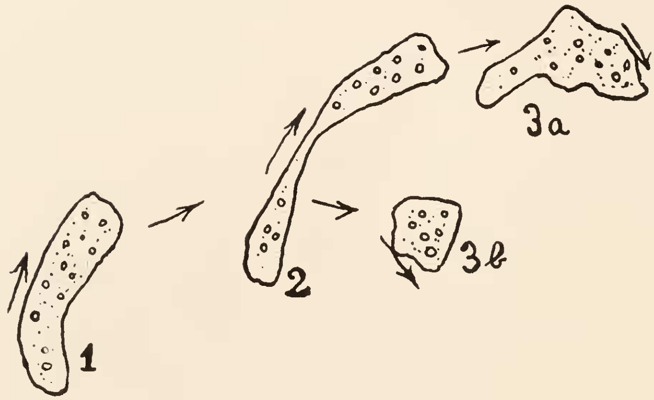

FIG. 2. Camera lucida drawings of a small amœba just before division (1) and (2) and immediately after division (3*a*) and (3*b*).

covered until the end of two months as stated. It is, therefore, evident that even one large amœba could not have remained in the large form unobserved during the growth period of nearly two months.

It is evident that the small forms develop into large ones and that as they do so they divide from time to time.

SUMMARY.

Individuals of *Amœba proteus* sometimes break up into from 100 to 300 amœboid forms. These amœboid forms gradually become larger until at the end of about two months they are as large as the original specimens. During the increase in size division occurs from time to time.

LITERATURE CITED.

Calkins, G. N.

'05 Evidences of a Sexual Cycle in the Life History of *Amœba proteus*. Archiv. f. Protistenk. Bd. 5, pp. 1–16.

'07 The Fertilization of *Amœba proteus*. BIOL. BULL., Vol. 13, pp. 219–230.

Carter, H. J.

'56 Notes on Fresh Water Infusoria of the Island of Bombay. Ann. and Mag. of Nat. Hist., S. 2, Vol. 18, pp. 115–132.

'63 On *Amœba princeps* and its Reproductive Cells. Ann. and Mag. of Nat. Hist., S. 3, Vol. 12, pp. 30–51.

Hausman, L. A.

'20 Life History of *Amœba proteus* Liedy. Biol. Bull., Vol. 38, pp. 340–352.

Hopkins, D. L.

'26 The Effect of Hydrogen Ion Concentration on Locomotion and Other Life-processes in *Amœba proteus*. Proc. of Nat. Acad. Sci., Vol. 12, pp. 311–315.

Metcalf, M. M.

'10 Studies upon *Amœba*. Jour. Exp. Zoöl., Vol. 9, pp. 301–331.

Schaeffer, A. A.

'26 Recent Discoveries in the Biology of *Amœba*. Quart. Review of Biol., Vol. I, pp. 95–118.

Scheel, C.

'99 Beitrage zur Fortpflanzung der Amoeben. Fertschrift zur Siebensigsten Geburtstage von Car von Kupffer, Jena. 569 pp.

Taylor, M.

'24 *Amœba proteus*: Some Observations on its Nucleus, Life-History and Culture. Quart. Jour. Micr. Sci., Vol. 69, pp. 119–143.

Wallich, G. C.

'63 Further Observations on the Distinctive Characters and Reproductive Phenomena of Amœban Rhizopods. Ann. and Mag. of Nat. Hist., S. 3, Vol. 12, pp. 329–337.

'63 On the Distinctive Characters, Habits, and Reproductive Phenomena of the Amœban Rhizopods. Ann. and Mag. of Nat. Hist., S. 3, Vol. 12, pp. 448–468.